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ELECTROPHORESIS IN HORIZONTAL POLYACRYLAMIDE GEL

Second Report: Separation of the Components of Human Serum at Different Intervals

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~~In~~ an earlier ~~article~~* ~~we~~ investigated the extent to which it was possible with a predetermined interval (interval from point of reference to pre-protein about 25 cm) to improve the separation of the components of normal human serum by alteration of the buffer composition and ionic strength, the gel concentration, and the degree of cross linkage. ~~In the present~~ article ~~we~~ shall attempt to show what influence the distance of migration has on the separation of the serum proteins.

Method

Test Substances for Electrophoresis

Normal human serum Hp 2-1, Gc 1-1 = "NHS"
Total α fraction of a normal human serum Hp 1-1, obtained by electrophoresis in polyvinyl chloride = "PVCE α Fr"
α_2 fraction of a normal human serum Hp 2-2, obtained by two-time electrophoresis in polyvinyl chloride = "PVCE α_2 Fr"

Electrophoresis in PAA Gel [= polyacrylamide gel]

A 5.5% (g/vol.) solution was prepared of cyano gum 41 in gel buffer (0.0550M tris - 0.00646M citric acid - 0.00268M NaOH - 0.00813M boric acid; pH 8.7) and to every 10 g of cyano gum 100 mg ammonium peroxide disulfate and 1000 mg β-dimethylaminopropionitrile added while stirring.

The electrophoresis chambers used were of varying width and length, but of a constant height of 3 mm. After the chamber was opened and the agar strip removed, protein-impregnated filter papers (Schleicher & Schüll No. 2315) were laid on the sticky place. The field strength applied was raised from 2-3 v/cm at the beginning of electro-

*Zwisler, O. and Biel, H., *Z.Klin.Chem.u.Klin.Biochem.*, Vol 4, 1966, page 58.

Table 1. Number of Protein Components as a Function of the Distance of Migration

Distance of Migration of the Prealbumin	Number of Protein Components								
	Human Serum			PVCA α Fraction			PVCA α_2 Fraction		
	Prealbumin Transferrin	Posttransferrin	Total No.	Prealbumin Transferrin	Posttransferrin	Total No.	Prealbumin Transferrin	Posttransferrin	Total No.
5 cm (Microgel = Fig.1,A)	10	11	21	9	10	19	6	12	18
9 cm (Semimicrogel = Fig.1,B)	10	11	21	9	9	18	9	12	21
27 cm (Macrogel = Fig. 1,C)	12	16	28	14	15	29	15	15	30
63 cm (Ultramicrogel = Fig. 1,D)	16	20	36	20	15	35	18	19	37

GRAPHIC NOT REPRODUCIBLE

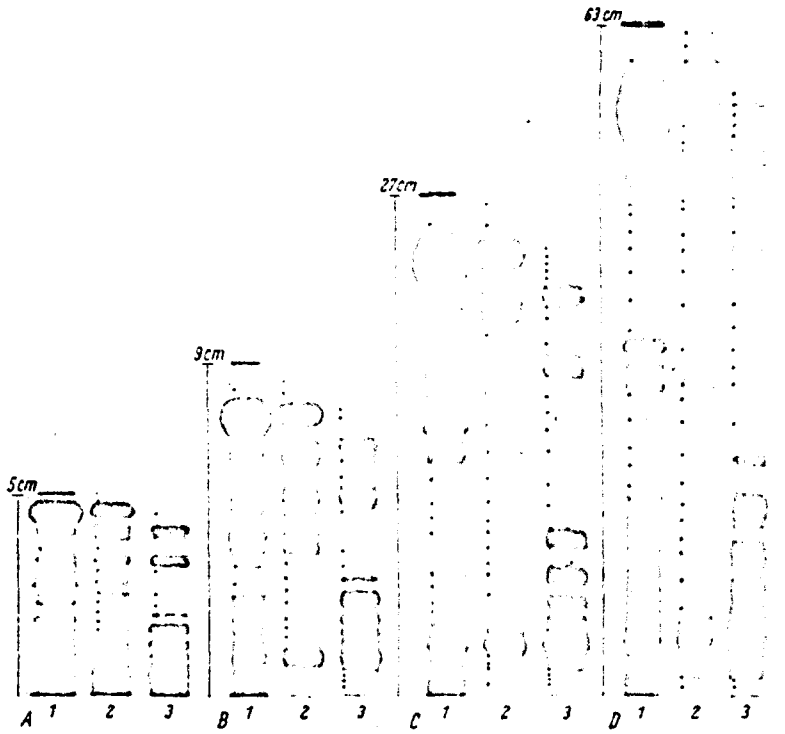

Figure 1. Electrophoresis of 1 = normal human serum, 2 = PVCE α Fr, and 3 = PVCE α_2 Fr in A = microgel, B = semimicrogel, C = macrogel, and D = ultramacrogel. Plotted at A: 175 - 70 - 70 µg
B: 525 - 210 - 210 µg
C: 1050 - 420 - 420 µg
D: 3150 - 1250 - 1250 µg protein per cm.

phoresis to 7-8 v/cm after about 2 hours. (The low field strength at the beginning of the electrophoresis probably promotes the migration of the proteins into the gel.) The connection to the electrode vessels was accomplished by means of filter papers impregnated with electrode buffers (0.12M NaOH - 0.6M boric acid). The Joulean heat which developed was sometimes conducted away by laying a water-soaked filter paper on the electrophoresis chamber (transpiration cooling). The running time was 3-4 hours for the semimicrogel 14 cm in length, 15-17 hours for the macrogel 34 cm in length, and 24-28 hours for the ultramacrogel 74 cm in length.

Results and Discussion

It is to be expected that with a longer migration distance the mixtures of components which with a short migration distance still appear homogeneous will be broken down by the filter effect of the PAA gel. For that reason we carried out experiments with human serum and two fractions obtained from it on gels 8, 14, 34, and 74 cm in length. The distance of the prealbumin from the starting point was 5, 9, 27, and 63 cm respectively. In a comparison of the images of the electrophoresis migrations (Figure 1) the most striking feature is the increasing sub-

division in the postalbumin range in the gels with the greater distance of migration. The number of components, as Table 1 shows, is about the same in fractionation in the microgel (= 5 cm) and semimicrogel (= 9 cm) and rises in the case of the ultramacrogel (= 63) to about double. On the average the subdividing in the prealbumin transferrin range increases somewhat more than that of the posttransferrin range. As compared to the microgel with 8 components in the prealbumin-transferrin range and 11 in the posttransferrin range, the macrogel shows on the average 14 to 15 and the ultramacrogel sometimes 18 bands.

In the light of these results it is desirable to use PAA gel electrophoreses with a short migration distance (distance from prealbumin to starting point up to 10 cm) as far as possible only for preliminary tests, but for tests of purity, especially where there are only slight differences in the rates of migration, to maintain migration distances of not less than 25 cm.

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